

Rat 2891-2894

(Back 14)

McAb to N1053

humorin N1053 CT: CR(NH)(Om) SLEMSAL/Thyro started 4/20/93

① 1<sup>st</sup> immunization IP  $\bar{c}$  FCA

Note: 0.8 ml aqueous  
+ 0.8 ml FCA

1.0 ml total, then 0.4 ml / rat.

② 2<sup>nd</sup> immunization IP  $\bar{c}$  FCA 5-12-93

③ 3<sup>rd</sup> IP  $\bar{c}$  FCA 6-3-93

④ Test bleed from ~~tail~~ Tail 6-9-93

⑤ 4<sup>th</sup> IP  $\bar{c}$  FCA JC/AF 6-22-93

⑥ Rats # 2893 + 2894 were boosted IP + intrasplenic  
~~0.5 ml  $\Rightarrow$  200  $\mu$ g N1053/Thyro conjugate #2~~

0.5 ml  $\Rightarrow$  200  $\mu$ g N1053/Thyro. conjugate #2  
per rat; sterile filtered & dissolved in PBS

⑦ r 2893 - Term. A/S 7-3-93

⑧ r 2894 - Term. A/S 7-6-93

⑨ 5<sup>th</sup> IP  $\bar{c}$  FCA RW/JC 8-10-93

⑩ Test Bleed from Tail r2891 + r2892 RW 9/2/93

⑪ r2891 and r2892 boosted IP + intrasplenic IV, final test prior RW/JC 9/14/93  
to fusion

⑫ r2891 and r2892 - Termined by cervical dislocation, de. 9/17/93  
euphoric, then blood was collected. Spleens dissected out for fusion RW/JC



# EASY BEAM

Plate # 1 N053 rats Date 6 / 18 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment \_\_\_\_\_

		1	2	3	4	5	6	7	8	9	10	11	12	
r2891	A	+0.071	+1.526	+1.470	+1.289	+1.102	+0.896	+0.614	+0.351	+0.238	+0.122	+0.114	+0.081	A IgG
r2891	B	-0.005	+0.335	+0.170	+0.093	+0.048	+0.025	+0.011	+0.000	+0.000	-0.009	-0.009	-0.011	B IgM
r2892	C	+0.078	+1.424	+1.396	+1.218	+1.034	+0.847	+0.594	+0.407	+0.285	+0.175	+0.101	+0.094	C IgG
r2892	D	+0.000	+1.325	+0.956	+0.585	+0.338	+0.186	+0.089	+0.043	+0.014	+0.000	+0.000	+0.000	D IgM
r2893	E	+0.582	+1.822	+1.841	+1.763	+1.813	+1.771	+1.640	+1.531	+1.380	+1.218	+0.896	+0.623	E IgG
r2893	F	+0.000	+1.779	+1.377	+0.765	+0.372	+0.194	+0.094	+0.049	+0.020	+0.005	+0.000	+0.000	F IgM
r2894	G	+0.159	+1.671	+1.623	+1.417	+1.194	+0.968	+0.672	+0.426	+0.285	+0.205	+0.200	+0.124	G IgG
r2894	H	+0.000	+0.527	+0.291	+0.173	+0.089	+0.042	+0.019	+0.006	+0.000	+0.000	+0.000	+0.000	H IgM
		blank	100	200	400	800	1600	3200	6400	12800	25600	51200	102400	

## NO 53 FUSION

7/6/93

Y3.112XT-75

Harvested as separate groups floating and attached cells

Cell Counts and Viability: Estimates

1. Floating  $94\% \times (10) \times 10^4 = 9.4 \times 10^6 / \text{ml}$  $9.4 \times 10^6 / \text{ml} (-36 \text{ ml})$  $\approx 3.38 \times 10^8$  cells total - viable $94/95$  alive =  $98.9\%$  viability

2. Attached

 $113 \times 10^6$  cells (124) =  $1.40 \times 10^8$  cells $\approx 96.5\%$  viability

113 alive / 115 total

(Many still attached to bottom of flask, difficult to remove)

Splenoocytes (Rat)

Spleen #1 (Rat #2693):

Pool A =  $261 @ 29 \text{ ml}$ Pool B =  $317 @ 29 \text{ ml}$  $5.78 \times 10^4$  cells =  $5.78 \times 10^6$  cells (29 ml) $= 1.67 \times 10^8$  cells

Spleen #2 (Rat 2694):

 $2.59 \times 10^4 \times 45 = 1.16 \times 10^8$  cells+  $\sim 20\%$  extra from another tube  $\frac{.30 \times 10^8 \text{ cells}}{1.46 \times 10^8 \text{ cells}}$ Total splenoocytes =  $1.67 \times 10^8 + 1.46 \times 10^8 \approx 3 \times 10^8$  splenoocytes totalRatio of splenoocytes: Y3.1 = 2:1  $\therefore$  use:1)  $3 \times 10^8$  splenoocytes2)  $1.5 \times 10^8$  Y3.1Y3.1

2nd count after spin down 2x:

 $514 \times 10^4 \times 44 \text{ ml} = 2.6 \times 10^8$  Y3 total $1.5 \times 10^8$  cells = 25 ml stock

# Screening Master Plates

## NOS3 fusion

### EASY BEAM

Plate # 1  
 Operator DF/KF

Date 8/5/93 Filter 492 nm  
 Comment NOS3 AT 100 ng/well

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.029	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	A
B	+0.008	+0.000	+0.026	+0.021	+0.013	+0.000	+0.000	+0.000	+0.006	+0.006	+0.006	+0.000	B
C	+0.007	+0.007	+0.007	+0.007	+0.007	+0.025	+0.009	+0.009	+0.014	+0.007	+0.000	+0.007	C
D	+0.000	+0.000	+0.000	+0.000	+0.014	+0.022	+0.006	+0.006	+0.015	+0.000	+0.008	+0.008	D
E	+0.000	+0.000	+0.000	+0.000	-0.005	+0.022	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	E
F	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	+0.000	+0.000	+0.000	+0.000	+0.005	+0.007	+0.007	+0.007	+0.007	+0.013	+0.011	+0.006	G
H	+0.000	+0.000	+0.000	+0.000	+0.006	+0.006	+0.010	+0.010	+0.005	+0.008	+0.006	+0.006	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate #

2

Operator

DF/KF

Date

8 / 5 / 93

Filter

492

nm

Comment

NAS3 @ 100ug/well

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.009	+0.009	+0.009	+0.011	+0.009	+0.000	-0.010	-0.006	-0.010	-0.005	A
B	-0.012	-0.012	-0.007	-0.012	-0.010	-0.010	-0.006	-0.010	-0.010	+0.000	-0.005	-0.010	B
C	-0.017	-0.011	-0.007	+0.000	-0.011	-0.011	-0.011	-0.011	-0.011	-0.011	-0.009	-0.009	C
D	-0.009	-0.012	-0.007	-0.013	-0.008	-0.006	-0.016	-0.016	-0.016	-0.009	-0.011	-0.009	D
E	-0.012	-0.012	-0.010	+0.000	+0.009	+0.014	-0.014	-0.019	-0.013	-0.007	-0.013	+0.000	E
F	+0.012	+0.000	-0.016	-0.016	-0.016	-0.013	-0.015	-0.015	-0.015	-0.013	-0.013	-0.018	F
G	-0.014	+0.008	-0.013	-0.017	-0.013	-0.010	-0.013	-0.016	-0.018	-0.013	-0.011	-0.013	G
H	-0.022	-0.016	-0.014	-0.014	-0.014	-0.014	-0.005	-0.018	-0.018	-0.018	-0.013	-0.013	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # 3 Date 8/5/93 Filter 492 nm  
 Operator DF/KF Comment N053@ 100 ng/well

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.005	+0.009	+0.007	+0.012	+0.008	+0.008	+0.011	+0.007	+0.011	A
B	+0.011	+0.009	+0.009	+0.009	+0.009	+0.009	+0.009	+0.011	+0.011	+0.015	+0.015	+0.008	B
C	+0.006	+0.010	+0.010	+0.008	+0.008	+0.011	+0.009	+0.011	+0.009	+0.009	+0.013	+0.007	C
D	+0.007	+0.009	+0.009	+0.009	+0.000	+0.025	+0.010	+0.012	+0.007	+0.007	+0.010	+0.010	D
E	+0.008	+0.006	+0.000	+0.000	+0.019	+0.031	+0.013	+0.011	+0.020	+0.007	+0.011	+0.011	E
F	+0.011	+0.011	+0.011	+0.007	+0.010	+0.040	+0.020	+0.013	+0.016	+0.016	+0.012	+0.014	F
G	+0.020	+0.006	+0.029	+0.022	+0.017	+0.007	+0.007	+0.015	+0.013	+0.009	+0.013	+0.013	G
H	+0.006	+0.006	+0.008	+0.036	+0.010	+0.000	+0.000	+0.008	+0.000	+0.009	+0.018	+0.008	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 # 4  
OperatorDate 8 / 5 / 93  
Comment N053 screening Assy

Filter \_\_\_\_\_ nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.010	+0.012	+0.005	+0.009	+0.009	+0.000	-0.010	+0.012	+0.000	+0.005	A
B	+0.000	+0.007	+0.007	+0.033	+0.024	+0.027	+0.016	+0.010	+0.000	+0.000	+0.000	+0.000	B
C	+0.012	-0.006	+0.014	+0.010	+0.000	+0.018	+0.000	+0.006	-0.007	+0.000	-0.006	+0.000	C
D	+0.012	+0.000	+0.017	+0.000	+0.000	+0.000	+0.005	+0.000	+0.010	-0.005	+0.000	+0.000	D
E	+0.014	+0.010	+0.007	+0.018	+0.029	+0.007	+0.000	+0.005	+0.020	+0.000	-0.006	+0.005	E
F	+0.017	+0.006	+0.006	+0.017	+0.017	+0.038	+0.010	+0.012	+0.030	+0.005	+0.000	+0.009	F
G	+0.016	+0.025	+0.010	+0.017	+0.000	+0.024	+0.000	+0.000	+0.000	+0.009	+0.000	+0.012	G
H	+0.017	+0.020	+0.010	+0.022	+0.000	+0.010	+0.014	+0.011	+0.000	+0.000	+0.010	+0.013	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 # 5  
OperatorDate 8 / 5 / 93  
Comment N053 Screening Assay

Filter \_\_\_\_\_ nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.007	+0.000	-0.006	+0.010	+0.000	+0.000	+0.029	-0.005	+0.000	+0.000	-0.007	A
B	+0.024	+0.028	+0.013	+0.000	+0.006	-0.010	+0.000	+0.000	-0.010	+0.000	+0.000	+0.000	B
C	+0.000	+0.000	+0.014	-0.006	+0.000	+0.009	-0.006	-0.006	+0.007	-0.005	+0.000	+0.000	C
D	+0.022	+0.006	+0.000	+0.012	+0.010	+0.000	-0.005	+0.011	-0.008	+0.005	+0.012	+0.000	D
E	+0.005	+0.027	+0.008	+0.010	+0.020	+0.022	+0.012	+0.000	+0.000	+0.012	+0.029	+0.000	E
F	+0.006	+0.010	+0.006	+0.008	+0.013	+0.025	+0.016	+0.000	+0.008	+0.000	+0.000	+0.006	F
G	+0.000	+0.005	+0.009	+0.005	+0.022	+0.016	+0.010	+0.000	+0.009	+0.000	+0.000	+0.010	G
H	+0.000	+0.029	+0.037	+0.034	+0.016	+0.025	+0.000	+0.008	+0.020	+0.005	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 #6 Date 8 / 5 / 93 Filter \_\_\_\_\_ nm  
Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.008	+0.006	+0.000	+0.007	+0.000	<u>+0.129</u>	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	A
B	+0.006	+0.015	+0.015	+0.000	+0.000	-0.009	+0.011	-0.008	-0.008	-0.008	+0.000	+0.014	B
C	+0.012	+0.008	+0.033	+0.000	+0.008	+0.000	+0.000	-0.005	+0.006	-0.008	+0.000	+0.010	C
D	+0.007	+0.007	+0.000	+0.010	+0.000	+0.000	+0.000	-0.005	-0.005	+0.000	+0.000	+0.000	D
E	+0.000	+0.005	+0.017	+0.005	+0.016	+0.000	+0.012	+0.005	+0.014	+0.000	+0.000	+0.000	E
F	+0.014	+0.012	+0.021	+0.027	+0.018	+0.018	+0.013	+0.000	+0.013	+0.000	+0.000	+0.006	F
G	+0.014	-0.005	+0.018	+0.008	+0.000	+0.014	+0.033	+0.000	+0.005	+0.008	+0.000	+0.000	G
H	<u>+0.053</u>	+0.006	+0.009	+0.019	+0.040	+0.013	+0.000	+0.000	-0.005	+0.006	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

# EASY BEAM

Plate # N053 #7 Date 8 / 5 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.019	+0.008	+0.016	+0.009	+0.000	+0.013	+0.017	+0.007	+0.005	+0.007	+0.009	+0.000	A
B	+0.000	+0.026	+0.019	+0.014	+0.000	+0.029	+0.011	+0.007	+0.011	+0.013	+0.009	+0.006	B
C	+0.009	+0.024	+0.006	+0.008	+0.010	+0.012	+0.017	+0.024	+0.008	+0.008	+0.026	+0.008	C
D	+0.012	+0.016	+0.019	+0.019	+0.038	+0.026	+0.040	+0.012	+0.016	+0.008	+0.015	+0.015	D
E	+0.008	+0.010	+0.013	+0.010	+0.016	+0.016	+0.018	+0.016	+0.048	+0.030	+0.025	+0.010	E
F	+0.035	+0.019	+0.009	+0.005	+0.026	+0.028	+0.021	+0.019	+0.019	+0.021	+0.012	+0.016	F
G	+0.000	+0.016	+0.032	+0.008	+0.023	+0.031	+0.027	+0.030	+0.018	+0.008	+0.027	+0.016	G
H	+0.026	+0.009	+0.012	+0.012	+0.012	+0.024	+0.033	+0.025	+0.014	+0.017	+0.020	+0.031	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 #8 Date 8 / 5 / 92 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.013	-0.008	+0.008	+0.005	+0.000	+0.026	+0.000	+0.000	+0.000	+0.000	+0.000	-0.011	A
B	+0.019	-0.018	+0.000	+0.025	+0.011	+0.011	+0.000	+0.000	+0.019	-0.008	-0.008	+0.000	B
C	+0.015	-0.011	+0.007	-0.009	+0.000	+0.000	+0.043	+0.000	+0.000	+0.000	+0.000	-0.005	C
D	+0.017	+0.000	-0.008	+0.019	+0.016	-0.007	+0.005	+0.009	+0.013	+0.017	-0.008	-0.005	D
E	+0.009	+0.000	+0.010	+0.000	+0.023	+0.000	+0.000	+0.000	+0.000	-0.008	-0.005	+0.000	E
F	+0.000	+0.000	+0.019	+0.013	+0.006	+0.012	-0.006	+0.011	+0.009	+0.000	+0.000	-0.010	F
G	+0.074	+0.000	+0.006	+0.000	+0.010	-0.005	+0.007	+0.000	+0.000	+0.000	-0.014	+0.027	G
H	+0.023	+0.033	+0.010	+0.005	+0.000	+0.000	+0.010	+0.007	+0.005	+0.000	-0.010	-0.008	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 #9 Date 8 / 5 / 93 Filter \_\_\_\_\_ nm  
Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.038	-0.007	+0.008	+0.000	+0.000	+0.010	-0.008	-0.008	+0.000	+0.000	-0.008	+0.000	A
B	+0.007	+0.013	+0.009	+0.017	+0.015	-0.006	-0.006	+0.000	+0.008	-0.010	-0.010	+0.000	B
C	+0.007	+0.019	+0.010	+0.006	+0.021	+0.000	+0.000	+0.000	+0.000	-0.012	-0.010	-0.010	C
D	+0.005	+0.009	+0.019	+0.011	+0.016	+0.000	+0.006	+0.049	+0.006	+0.000	-0.005	+0.000	D
E	+0.000	+0.015	+0.021	+0.033	+0.005	+0.005	+0.005	+0.005	+0.024	-0.011	+0.011	-0.010	E
F	+0.062	+0.023	+0.031	+0.046	+0.017	+0.045	+0.055	+0.000	+0.000	-0.008	-0.006	+0.000	F
G	+0.051	+0.016	+0.016	+0.021	+0.013	+0.013	+0.000	+0.008	+0.013	+0.000	-0.010	+0.008	G
H	+0.008	+0.000	+0.022	+0.018	+0.007	+0.043	+0.026	+0.011	+0.018	+0.005	+0.020	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

# EASY BEAM

Plate # N053 #11 Date 6 / 5 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.012	-0.007	+0.000	+0.000	+0.000	-0.007	+0.016	+0.000	-0.006	-0.010	-0.005	+0.017	A
B	+0.000	+0.000	+0.009	+0.016	+0.014	+0.000	+0.031	-0.008	-0.008	+0.000	+0.015	+0.000	B
C	+0.000	+0.027	+0.000	+0.000	+0.016	+0.024	+0.054	-0.005	+0.006	-0.007	+0.007	+0.007	C
D	+0.009	+0.012	+0.000	+0.007	+0.027	+0.010	+0.019	+0.006	-0.017	-0.017	-0.010	-0.005	D
E	+0.030	+0.000	+0.009	+0.006	+0.006	+0.000	+0.034	+0.005	+0.065	-0.015	+0.005	+0.053	E
F	+0.006	+0.000	+0.000	+0.019	+0.019	+0.038	+0.065	+0.000	-0.014	-0.007	+0.000	-0.012	F
G	+0.009	+0.016	-0.010	+0.000	+0.013	-0.009	+0.007	-0.009	+0.008	-0.016	+0.000	+0.000	G
H	+0.000	+0.026	+0.000	+0.000	-0.006	-0.006	+0.019	+0.000	+0.000	-0.009	+0.000	-0.010	H
	1	2	3	4	5	6	7	8	9	10	11	12	

# EASY BEAM

Plate # N053-IgG Date 8 / 11 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Titration Assay

	1	2	3	4	5	6	7	8	9	Blank			
	646	641	661	9F1	9F7	961	11C7	11E12	11F7	10	11	12	
A	+0.026	+0.017	+0.000	+0.034	+0.014	+0.007	+0.028	+0.000	+0.008	+0.038	+0.040	+0.040	A 1:2
B	+0.032	+0.013	+0.022	+0.006	+0.000	+0.009	+0.005	+0.005	+0.000	+0.036	+0.044	+0.044	B 1:4
C	+0.013	-0.008	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.054	+0.038	+0.052	C 1:8
D	+0.015	+0.000	+0.009	+0.005	+0.000	+0.017	+0.005	+0.046	+0.012	+0.043	+0.038	+0.065	D 1:16
E	+0.010	+0.000	+0.018	+0.020	+0.006	+0.000	+0.018	+0.000	+0.012	+0.054	+0.062	+0.049	E 1:32
F	+0.008	+0.010	+0.007	+0.007	+0.005	+0.000	+0.000	+0.000	+0.000	+0.047	+0.056	+0.042	F 1:64
G	+0.000	+0.011	+0.000	+0.014	+0.018	+0.014	+0.000	-0.009	+0.011	+0.079	+0.046	+0.042	G 1:128
H	+0.009	+0.005	+0.000	+0.012	+0.035	+0.018	+0.020	+0.007	+0.014	+0.048	+0.037	+0.046	H 1:256
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # NO53 - IgM Date 8 / 11 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment NO53 Titration Assay

										Blank			
	1	2	3	4	5	6	7	8	9	10	11	12	
	6A6	6H1	8G1	9F1	9F7	961	11C7	11E12	11F7				
A	+0.000	+0.000	+0.015	+0.011	+0.020	+0.000	+0.000	+0.036	-0.006	+0.000	+0.000	+0.000	A 1: 2
B	+0.000	+0.000	+0.009	+0.000	+0.009	+0.000	+0.000	+0.019	+0.000	+0.000	+0.000	+0.000	B 1: 4
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	-0.005	+0.000	+0.000	C 1: 8
D	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	D 1: 16
E	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	E 1: 32
F	+0.000	+0.000	+0.000	+0.000	+0.007	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	F 1: 64
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	G 1: 128
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	H 1: 256
	1	2	3	4	5	6	7	8	9	10	11	12	

## NO53 FUSION

Y21

Viable Cell count  $\rightarrow$  Y21 harvested by drawing media into pipet then dislodging cells adherent to flask w/ a stream of media

- Pool A  $205 \times 10^4$  cells/ml (40 ml) =  $8.2 \times 10^7$  cells  
 B  $186 \times 10^4$  cells/ml (45 ml) =  $8.3 \times 10^7$  cells  
 C  $330 \times 10^4$  cells/ml (35 ml) =  $1.16 \times 10^8$  cells  
 D  $304 \times 10^4$  cells/ml (35 ml) =  $1.06 \times 10^8$  cells  
 E  $8.75 \times 10^7$  cells

Total # Y21 =  $4.75 \times 10^8$  cells in 10 ml @ 95.8% avg viability

Splernocytes

- Count A  $210 \times 10^4$  cells (10 fold dilution) (25 ml) =  $5.21 \times 10^8$  splernocytes  
 B  $104 \times 10^4$  (10 fold dilution) (25 ml) =  $4.1 \times 10^8$  splernocytes

$$\bar{x} = \frac{4.6}{2} \times 10^8 \text{ splernocytes}$$

$\Rightarrow$  At 2:1 ratio of splern: myeloma (Y21)

$\Rightarrow$  Need  $\sim 2.05 \times 10^8$  Y21  $\Rightarrow$  4.34 ml  $\Rightarrow$  use  $\approx$  5 ml

Monday 9/30/93

SP2/0-Ag 14

2XTE75 picked off cells from back of flask then counted

Flask B  $105 \times 10^4$  cells/ml (1 ml) =  $1.53 \times 10^7$  cells

Flask C  $10 \times 10^4$  cells/ml (1 ml) =  $1.44 \times 10^7$  cells

$\bar{x}$  Viability = 92.5%

2XTE75 added 1 ml media

Media: DMEM 0.2% FCS 2.1% DMSO

Y21

2XTE75 added 1 ml media to each flask then media drawn on Wed 10/1

# EASY BEAM

Plate # N053 rats Date 9 / 3 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Rats-- 2<sup>nd</sup> Test Bleed  
Titration Assay --KF

		1	2	3	4	5	6	7	8	9	10	11	12	
r2891	A	+0.057	+1.564	+1.601	+1.268	+1.050	+0.843	+0.511	+0.329	+0.245	+0.173	+0.117	+0.109	A
r2892	B	+0.079	+1.891	+1.860	+1.740	+1.780	+1.653	+1.491	+1.246	+0.957	+0.675	+0.403	+0.322	B
r2891	C	+0.012	+1.085	+0.802	+0.529	+0.338	+0.192	+0.108	+0.062	+0.040	+0.029	+0.020	+0.017	C
r2892	D	+0.008	+1.936	+1.428	+0.807	+0.426	+0.244	+0.130	+0.074	+0.045	+0.029	+0.021	+0.017	D
	E	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	E
	F	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	F
	G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
	H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
		1	2	3	4	5	6	7	8	9	10	11	12	
		Blank	1→100	1→200	1→400	1→800	1→1600	1→3200	1→6400	1→12800	1→25600	1→51200	1→104200	

IgG

IgM

# EASY BEAM

Plate # NO53#1      Date 10 / 12 / 93      Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_      Comment ELISA Screen On NO53

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.010	+0.041	+0.043	+0.007	+0.000	0.007	-0.009	+0.000	-0.006	+0.000	+0.000	+0.013	A
B	+0.010	+0.022	+0.041	+0.000	+0.015	+0.015	+0.018	+0.044	+0.022	+0.036	+0.046	+0.000	B
C	+0.007	+0.092	+0.034	+0.000	+0.000	+0.019	+0.037	+0.009	+0.035	+0.000	+0.031	+0.031	C
D	+0.039	+0.010	+0.071	+0.014	+0.017	+0.037	+0.046	+0.009	+0.046	+0.000	+0.031	+0.050	D
E	+0.033	+0.009	+0.052	+0.027	+0.005	+0.021	+0.000	+0.007	+0.000	+0.000	+0.016	+0.365	E
F	+0.025	+0.009	+0.017	+0.021	+0.035	+0.022	+0.000	+0.036	+0.014	+0.000	+0.031	+0.005	F
G	+0.000	+0.015	+0.008	+0.017	+0.000	+0.015	+0.007	+0.013	+0.005	+0.012	-0.013	+0.020	G
H	+0.000	+0.015	+0.058	+0.064	+0.009	+0.041	+0.012	+0.012	+0.057	+0.000	+0.033	+0.044	H
	1	2	3	4	5	6	7	8	9	10	11	12	

1E12

# EASY BEAM

Plate # N053 #2 Date 10 / 12 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.025	+0.000	+0.000	+0.019	+0.000	0.009	+0.000	+0.048	+0.006	+0.000	+0.007	+0.000	A
B	-0.011	-0.000	+0.053	+0.005	+0.000	+0.035	-0.006	+0.020	+0.000	+0.036	+0.042	+0.005	B
C	+0.014	+0.000	+0.000	+0.006	+0.026	+0.006	+0.017	+0.000	+0.000	+0.009	+0.009	+0.007	C
D	+0.017	+0.042	+0.026	+0.011	+0.008	+0.014	+0.017	+0.000	+0.008	+0.005	+0.091	-0.007	D
E	+0.032	+0.012	+0.012	+0.012	+0.012	+0.010	+0.005	-0.470	+0.015	+0.000	+0.009	+0.041	E
F	-0.005	+0.012	+0.007	+0.018	+0.000	+0.026	-0.005	+0.011	+0.000	+0.013	+0.018	+0.000	F
G	-0.008	-0.005	+0.018	+0.018	+0.016	+0.000	+0.000	+0.000	+0.000	-0.012	+0.010	-0.006	G
H	-0.076	+0.017	+0.000	+0.012	+0.008	+0.000	+0.023	+0.000	+0.000	+0.000	+0.000	+0.051	H

2 E 8

# EASY BEAM

Plate # N053 #3 Date 10 / 12 / 93 Filter \_\_\_\_\_ nm  
Operator \_\_\_\_\_ Comment \_\_\_\_\_

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.016	-0.010	-0.005	+0.023	+0.016	+0.000	+0.033	+0.012	+0.000	+0.000	+0.007	A
B	+0.005	+0.005	+0.000	-0.011	-0.011	-0.011	-0.015	+0.000	+0.010	+0.005	+0.012	+0.014	B
C	+0.006	+0.000	+0.000	-0.005	-0.008	+0.000	+0.007	+0.000	-0.007	+0.011	-0.015	-0.006	C
D	+0.060	+0.017	+0.009	-0.009	+0.037	+0.000	-0.006	+0.000	+0.006	-0.011	+0.000	+0.000	D
E	+0.010	+0.033	+0.000	-0.006	+0.010	-0.011	+0.000	-0.009	+0.000	+0.016	-0.009	-0.005	E
F	+0.000	+0.014	+0.000	+0.000	+0.005	+0.007	+0.000	+0.014	+0.016	+0.019	+0.000	+0.006	F
G	+0.013	+0.013	+0.017	-0.006	-0.006	+0.000	+0.000	+0.000	+0.019	+0.051	+0.013	+0.013	G
H	+0.021	+0.028	+0.008	+0.000	+0.000	+0.006	+0.000	+0.000	+0.032	+0.000	+0.000	+0.047	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # NO53 #4  
OperatorDate 10 / 12 / 93  
Comment NO53 screening Assay

Filter \_\_\_\_\_ nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.010	+0.012	+0.005	+0.009	+0.009	+0.000	-0.010	+0.012	+0.000	+0.005	A
B	+0.000	+0.007	+0.007	+0.033	+0.024	+0.027	+0.016	+0.010	+0.000	+0.000	+0.000	+0.000	B
C	+0.012	-0.006	+0.014	+0.010	+0.000	+0.018	+0.000	+0.006	-0.007	+0.000	-0.006	+0.000	C
D	+0.012	+0.000	+0.017	+0.000	+0.000	+0.000	+0.005	+0.000	+0.010	-0.005	+0.000	+0.000	D
E	+0.014	+0.010	+0.007	+0.018	+0.029	+0.007	+0.000	+0.005	+0.020	+0.000	-0.006	+0.005	E
F	+0.017	+0.006	+0.006	+0.017	+0.017	+0.038	+0.010	+0.012	+0.030	+0.005	+0.000	+0.009	F
G	+0.016	+0.025	-0.010	+0.017	+0.000	+0.024	+0.000	+0.000	+0.000	+0.009	+0.000	+0.012	G
H	+0.017	+0.020	-0.010	-0.022	+0.000	+0.010	+0.014	+0.011	+0.000	+0.000	+0.010	+0.013	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 # 5 Date 10 / 12 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.007	+0.000	-0.006	+0.010	+0.000	+0.000	+0.029	-0.005	+0.000	+0.000	-0.007	A
B	+0.024	+0.028	+0.013	+0.000	+0.006	-0.010	+0.000	+0.000	-0.010	+0.000	+0.000	+0.000	B
C	+0.000	+0.000	+0.014	-0.006	+0.000	+0.009	-0.006	-0.006	+0.007	-0.005	+0.000	+0.000	C
D	+0.022	+0.006	+0.000	+0.012	+0.010	+0.000	-0.005	+0.011	-0.008	+0.005	+0.012	+0.000	D
E	+0.005	+0.027	+0.008	+0.010	+0.020	+0.022	+0.012	+0.000	+0.000	+0.012	+0.029	+0.000	E
F	+0.006	+0.010	+0.006	+0.008	+0.013	+0.025	+0.016	+0.000	+0.008	+0.000	+0.000	+0.006	F
G	+0.000	+0.005	+0.009	+0.005	+0.022	+0.016	+0.010	+0.000	+0.009	+0.000	+0.000	+0.010	G
H	+0.000	+0.029	+0.037	+0.034	+0.016	+0.025	+0.000	+0.008	+0.020	+0.005	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

# EASY BEAM

Plate # N053 #6 Date 10 / 12 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.015	+0.013	+0.025	+0.035	+0.037	+0.008	+0.008	+0.006	+0.000	+0.015	+0.011	A
B	-0.007	+0.000	+0.021	+0.021	+0.000	+0.017	+0.012	+0.077	+0.007	-0.006	+0.022	+0.012	B
C	+0.018	+0.042	+0.016	+0.023	+0.007	+0.007	+0.000	+0.000	+0.016	-0.008	+0.033	+0.048	C
D	+0.015	+0.042	+0.047	+0.016	+0.024	+0.016	+0.037	+0.049	+0.039	+0.000	+0.036	+0.048	D
E	+0.020	+0.037	+0.020	+0.020	+0.000	+0.028	+0.025	+0.000	+0.017	+0.010	+0.006	+0.026	E
F	+0.000	+0.017	+0.025	+0.015	+0.007	+0.041	+0.015	+0.000	+0.042	+0.007	+0.005	+0.041	F
G	+0.027	+0.020	+0.000	+0.015	+0.018	+0.050	+0.010	-0.008	-0.008	+0.000	+0.044	+0.044	G
H	+0.000	+0.011	+0.000	+0.000	+0.011	+0.007	+0.000	-0.010	+0.283	+0.000	+0.041	+0.027	H
	1	2	3	4	5	6	7	8	9	10	11	12	

6 H 9

# EASY BEAM

Plate # N053 #7 Date 10 / 12 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.019	+0.008	+0.016	+0.009	+0.000	+0.013	+0.017	+0.007	+0.005	+0.007	+0.009	+0.000	A
B	+0.000	+0.026	+0.019	+0.014	+0.000	+0.029	+0.011	+0.007	+0.011	+0.013	+0.009	+0.006	B
C	+0.009	+0.024	+0.006	+0.008	+0.010	+0.012	+0.017	+0.024	+0.008	+0.008	+0.026	+0.008	C
D	+0.012	+0.016	+0.019	+0.019	+0.038	+0.026	+0.040	+0.012	+0.016	+0.008	+0.015	+0.015	D
E	+0.008	+0.010	+0.013	+0.010	+0.016	+0.016	+0.018	+0.016	+0.048	+0.030	+0.025	+0.010	E
F	+0.035	+0.019	+0.009	+0.005	+0.026	+0.028	+0.021	+0.019	+0.019	+0.021	+0.012	+0.016	F
G	+0.000	+0.016	+0.032	+0.008	+0.023	+0.031	+0.027	+0.030	+0.018	+0.008	+0.027	+0.016	G
H	+0.026	+0.009	+0.012	+0.012	+0.012	+0.024	+0.033	+0.025	+0.014	+0.017	+0.020	+0.031	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 #8 Date 10 / 12 / 92 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.013	-0.008	+0.008	+0.005	+0.000	+0.026	+0.000	+0.000	+0.000	+0.000	+0.000	-0.011	A
B	+0.019	-0.018	+0.000	+0.025	+0.011	+0.011	+0.000	+0.000	+0.019	-0.008	-0.008	+0.000	B
C	+0.015	-0.011	+0.007	-0.009	+0.000	+0.000	+0.043	+0.000	+0.000	+0.000	+0.000	-0.005	C
D	+0.017	+0.000	-0.008	+0.019	+0.016	-0.007	+0.005	+0.009	+0.013	+0.017	-0.008	-0.005	D
E	+0.009	+0.000	+0.010	+0.000	+0.023	+0.000	+0.000	+0.000	+0.000	-0.008	-0.005	+0.000	E
F	+0.000	+0.000	+0.019	+0.013	+0.006	+0.012	-0.006	+0.011	+0.009	+0.000	+0.000	-0.010	F
G	+0.074	+0.000	+0.006	+0.000	+0.010	-0.005	+0.007	+0.000	+0.000	+0.000	-0.014	+0.027	G
H	+0.023	+0.033	+0.010	+0.005	+0.000	+0.000	+0.010	+0.007	+0.005	+0.000	-0.010	-0.008	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 #9 Date 10 / 12 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.038	-0.007	+0.008	+0.000	+0.000	+0.010	-0.008	-0.008	+0.000	+0.000	-0.008	+0.000	A
B	+0.007	+0.013	+0.009	+0.017	+0.015	-0.006	-0.006	+0.000	+0.008	-0.010	-0.010	+0.000	B
C	+0.007	+0.019	+0.010	+0.006	+0.021	+0.000	+0.000	+0.000	+0.000	-0.012	-0.010	-0.010	C
D	+0.005	+0.009	+0.019	+0.011	+0.016	+0.000	+0.006	+0.049	+0.006	+0.000	-0.005	+0.000	D
E	+0.000	+0.015	+0.021	+0.033	+0.005	+0.005	+0.005	+0.005	+0.024	-0.011	+0.011	-0.010	E
F	+0.062	+0.023	+0.031	+0.046	+0.017	+0.045	+0.055	+0.000	+0.000	-0.008	-0.006	+0.000	F
G	+0.051	+0.016	+0.016	+0.021	+0.013	+0.013	+0.000	+0.008	+0.013	+0.000	-0.010	+0.008	G
H	+0.008	+0.000	+0.022	+0.018	+0.007	+0.043	+0.026	+0.011	+0.018	+0.005	+0.020	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

## EASY BEAM

Plate # N053 #10 Date 10 / 12 / 93 Filter \_\_\_\_\_ nm  
 Operator \_\_\_\_\_ Comment N053 Screening Assay

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.012	-0.007	+0.000	+0.000	+0.000	-0.007	+0.016	+0.000	-0.006	-0.010	-0.005	+0.017	A
B	+0.000	+0.000	+0.009	+0.016	+0.014	+0.000	+0.031	-0.008	-0.008	+0.000	+0.015	+0.000	B
C	+0.000	+0.027	+0.000	+0.000	+0.016	+0.024	+0.054	-0.005	+0.006	-0.007	+0.007	+0.007	C
D	+0.009	+0.012	+0.000	+0.007	+0.027	+0.010	+0.019	+0.006	-0.017	-0.017	-0.010	-0.005	D
E	+0.030	+0.000	+0.009	+0.006	+0.006	+0.000	+0.034	+0.005	+0.065	-0.015	+0.005	+0.053	E
F	+0.006	+0.000	+0.000	+0.019	+0.019	+0.038	+0.065	+0.000	-0.014	-0.007	+0.000	-0.012	F
G	+0.009	+0.015	-0.010	+0.000	+0.013	-0.009	+0.007	-0.009	+0.008	-0.016	+0.000	+0.000	G
H	+0.000	+0.026	+0.000	+0.000	-0.006	-0.006	+0.019	+0.000	+0.000	-0.009	+0.000	-0.010	H
	1	2	3	4	5	6	7	8	9	10	11	12	

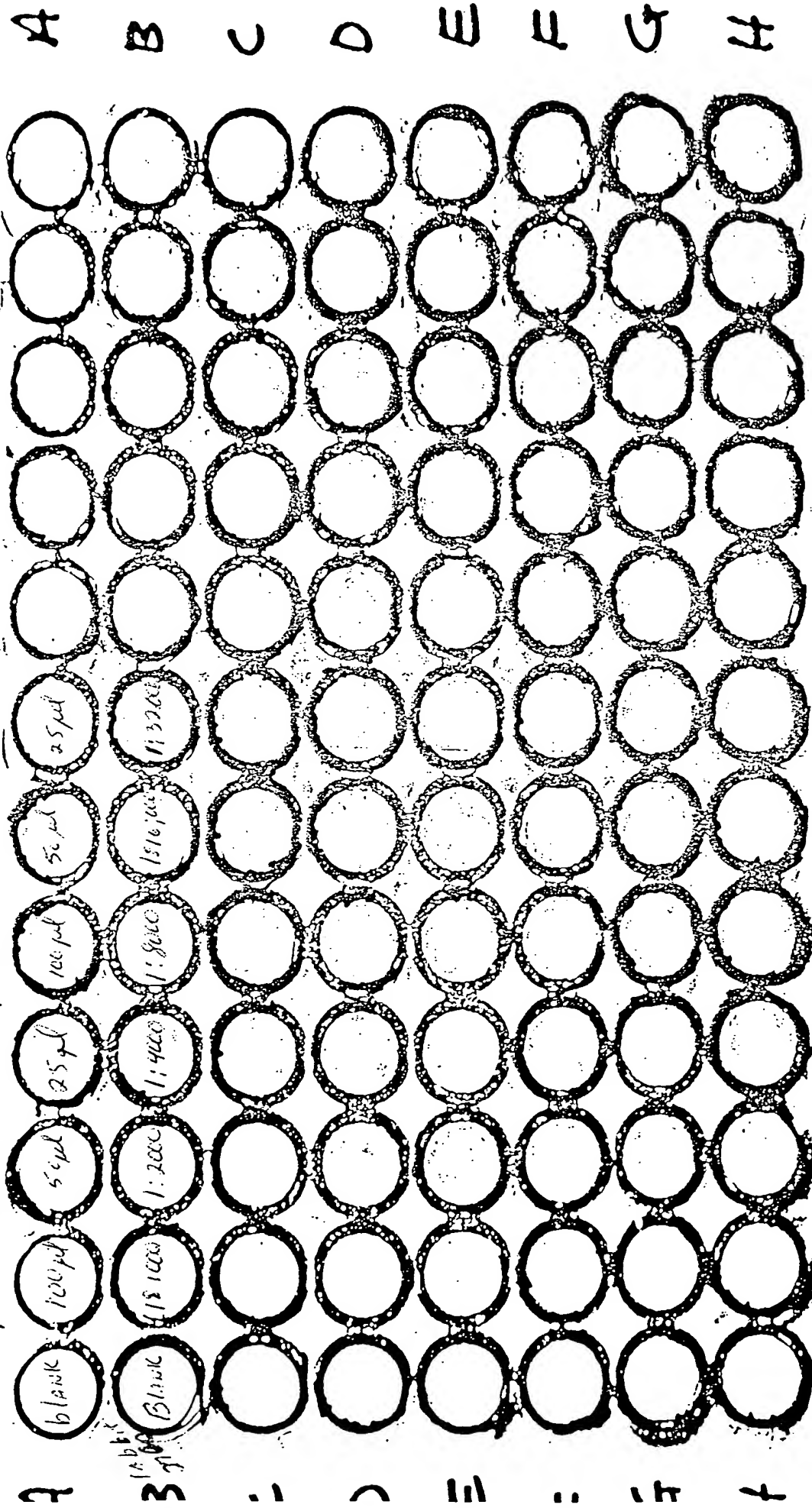
Testing Positive Hybrids for  
binding to ADP-Sepharose purified  
human ins

10/16/93

1 2 3 4 5 6 7 8 9 10 11 12

1E12 C.S.

2E8 C.S.



1 2 3 4 5 6 7 8 9 10 11 12

# EASY BEAM

Plate # rh, NOS Date 10 / 17 / 93 Filter 492 nm  
 Operator \_\_\_\_\_ Comment \_\_\_\_\_

Blank 1E12 C.S. 2E8 C.S.

1 100 µl 2 50 µl 3 25 µl 4 100 µl 5 50 µl 6 25 µl 7 100 µl 8 9 10 11 12

A	+0.000	+0.000	+0.000	+0.035	+0.007	+0.000	+0.000	=====	=====	=====	=====	=====	A
B	+0.000	+OVER	+OVER	+1.913	+1.297	+0.691	+0.311	=====	=====	=====	=====	=====	B
C	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	C
D	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	D
E	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	E
F	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	F
G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
	1	2	3	4	5	6	7	8	9	10	11	12	

No McAb binding!

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